PLANT DEVELOPMENT WORKSHOP #13

UNIVERSITY OF WESTERN ONTARIO, LONDON, NOVEMER 5, 1983

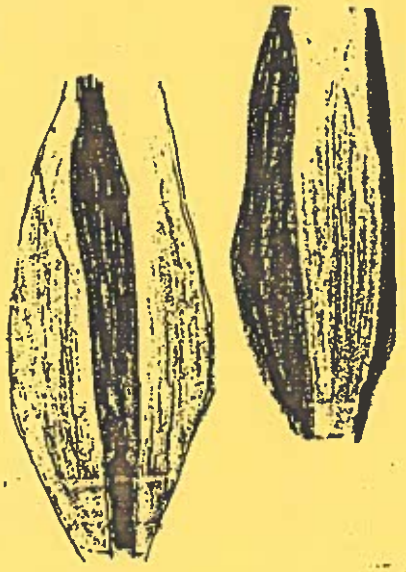
Program Schedule

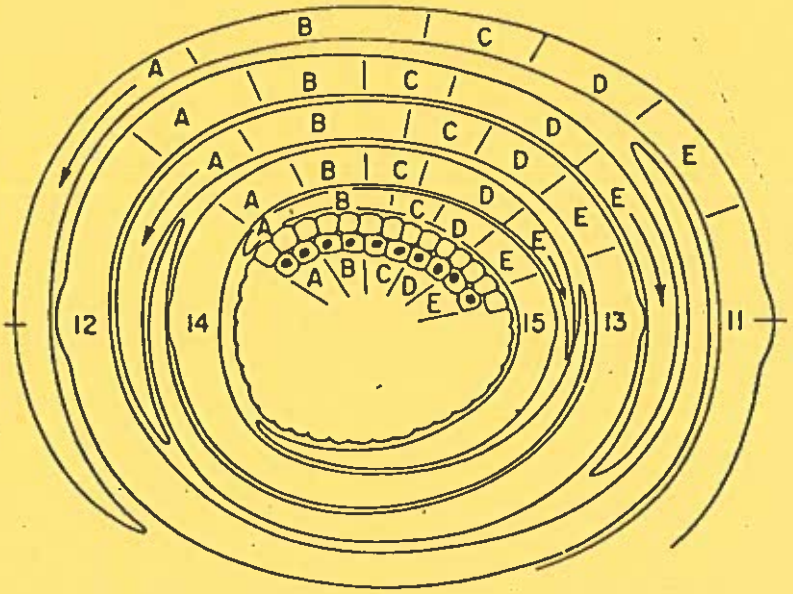
Time		
9:00 - 9:30 a.m.	Registration and Coffee	Room B&G 217
9:30 - 11:00 a.m.	Paper Presentations - Session A	Room B&G 22
11:00 - 11:30 a.m.	INTERMISSION - Coffee etc.	Room B&G 217
11:30 - 1:00 p.m.	Paper Presentations - Session B	Room B&G 22
	Session C	Room B&G 52
1:00 - 2:30 p.m.	LUNCH	Room B&G 217
2:30 - 4:00 p.m.	**Special Lecture**	

DR. Ed H. COE - Professor of Agronomy,
University of Missouri
Columbia, Missouri

Title: "Implications of Induced Sectors for Interpretations of Plant Development"

Stephanoon proposed sectors quantitatively from striped leaf





PAPER PRESENTATIONS

SESSION A

Room B&G 22 9:30 - 11:00 a.m.

Starting Time	
9:30 a.m.	R. N. CHIBBAR and R. B. VAN HUYSTEE (U.W.O.) The Role of Peroxidase in Cell Development
9:45 a.m.	Y. PICHE, R. L. PETERSON and C. A. ACKERLEY (Guelph) Localization of RNA in Corn Root Meristematic Cells During RNAase - Colloidal Gold Complexes: An Example of Protein Gold Labelling of Plant Tissues
10:00 a.m.	CAROL A. PETERSON and JANICE M. C. FISCHER (Waterloo) A New Test for Cell Vitality
10:15 a.m.	DAYAKER PAREDDY (U.W.O.) In vitro Culture of Field Corn Tassels
10:25 a.m.	BOMMINENI V. REDDY (U.W.O.) In vitro Culture of Corn Ears
10:35 a.m.	G. T. ROTUNDO, P. M. HARNEY and H. TIESSEN (Guelph) Poster Direct and Indirect Embryo genesis in Microspores of Asparagus efficinalis L. cv. "Viking"
10:50 a.m.	PIERRE FOBERT and D. WEBB (~~McMaster~~ Queens) Cytokinin Induced Shoot Formations With Lettuce Colyledons

SESSION B

Room B&G 22 11:30 a.m. - 1:00 p.m.

Starting Time	
11:30 a.m.	D. J. GRENVILLE and Y. PICHE (Guelph) Sclerotial Development in Two Ectomycorrhizal Fungi, *Pisolithos tinctorius* and *Paxillus involutus*
11:45 a.m.	FRANCES ALLEN (Toronto) Compatible and Incompatible Interactions Between Flax Rust and near Isogenic Lines of its Host
12:00 p.m.	A. WALTER and N. ROSA (Agri. Canada, Delhi) A Preliminary Report on Water Uptake by the Imbibing and Germinating Seed of Tobacco
12:15 p.m.	MARK BRUNDBETT, Y. PICHE and R. L. PETERSON (Guelph) A New Technique for Staining Vesicular Arbuscular Mycorahizae in Cleared Roots
12:30 p.m.	J.-P. ZHAO (McMaster) Mitotic Abnormalities Induced in Roots of *Z. mays* by MMS
12:45 p.m.	D. WILSON (McMaster) Lesions Induced by Maleic Hydrazide Continue to be Expressed over Several Cell cycles

SESSION C

Room B&G 52 11:30 a.m. - 1:00 p.m.

Starting Time	
11:30 a.m.	I. OCKENDEN (McMaster) Measurement of Calcium in *Curcurbita maxima* and *C. andreana* Embryos
11:45 a.m.	J. M. PETITTE and D. P. ORMROD (Guelph) Effects of Sulphur Dioxide and Nitrogen Dioxide on Intumescence Development of Leaves of *Solanum tuberosom*
12:00 p.m.	ROLF RUTISHAUSER (McGill) Organization of the "Leaf Whorls" of *Hydrothrix gardneri*
12:15 p.m.	CLAIRE COONEY-SOVETTS and ROLF SATTLER (McGill) Phylloclade Development in the Asparagaceae
12:30 p.m.	NANCY G. DENGLER, RONALD E. DENGLER and PAUL W. HATTERSLEY (Toronto) Differing Ontogenetic Origin of PCR ('Kranz') Sheaths in the Leaf Balde of C_4 grasses
12:45 p.m.	JACK MAZE (UBC) Morphometric Analysis of Ovule Development in *Nothofagus antarctica*

ABSTRACTS

Compatible and incompatible interactions between flax rust and near-isogenic lines of its host. Frances H.E.Allen, Botany Dept., Uni. of Toronto, Toronto, Ontario M5S 1A1.

Histological differences became evident in the leaves of eight near-isogenic lines of flax infected with race 1 of the rust fungus Melampsora lini. Compared with the compatible L^{9} and M^{1} interactions, fungal growth was progressively more restricted in the moderately incompatible K and M^{4}, incompatible M and P, and highly incompatible L and M^{3} interactions. This restriction preceded appreciable necrosis in K, M^{4}, M and P. However, in L and M^{3} host necrosis was more rapid and the fungus was restricted to a few host cells. An early ultrastructural event of incompatible interactions was the appearance of extensive fibrillar deposits in the extrahaustorial matrix. These deposits gave a positive reaction for silver proteinate indicating a carbohydrate component but were not extracted by cellulase, pectinase or chitinase. In incompatible interactions clusters of electron-dense particles sometimes replaced starch in plastids of infected host cells. This event coincided with the appearance of matrical deposits. In M and P these particles were also found in plastids of adjoining uninfected cells. They were extracted by pullulanase followed by α-amylase, indicating that they consisted of a highly branched amylopectin.

A new technique for staining vesicular-arbuscular mycorrhizae in cleared roots. Brundrett, Mark, Y. Piché and R.L. Peterson, Department of Botany and Genetics, University of Guelph, Guelph, Ontario.

A new staining procedure using chlorazol black E has been developed for staining vesicular-arbuscular mycorrhizal fungi in cleared roots. In a comparative study, chlorazol black E was found to be much superior to previously used stains (acid fuschsin, trypan blue, aniline blue) for showing details of internal hyphae and particularly arbuscules. This clearing and staining procedure, combined with Nomarski interference contrast microscopy, revealed details of arbuscule structure not evident with previous techniques. A survey of roots of several species of plants showed that differences in arbuscule morphology may be useful in the taxonomy of this group of fungi. The procedure should also allow for more accurate assessments of roots for degree of colonization by vesicular-arbuscular fungi.

Phylloclade Development in the Asparagaceae - Claire Cooney-Sovetts and Rolf Sattler. Department of Biology, Mc Gill University, Montreal, P.Q.

Phylloclades are traditionally defined as flattened, determinate, leaf-like stems primarily on the basis of their axillary position. Historically, however, the literature is replete with controversy over what the morphological nature of these organs is. In view of this, a study of phylloclade development in comparison with leaf and stem development was undertaken in four closely related species of the Asparagaceae: Ruscus aculeatus L., Danae racemosa Mönch, Semele androgyna Kunth and Asparagus sprengeri Regel. Results of SEM, epi-illumination and thin plastic sectioning techniques reveal a continuum in phylloclade development from very leaf-like forms such as those of Danae to more intermediate types such as the fertile and terminal phylloclades of Ruscus, to the gradually more shoot-like forms of Semele and Asparagus. In each species, however, a combination of shoot and leaf-like characteristics is seen, which when weighted equally, demonstrates that the phylloclade in this group is an intermediate organ, partially homologous to a leaf and a stem. As such, it is a form of evolutionary novelty and exemplifies the phenomenon of homeosis, which is the transference of characters from one organ to another.

R. N. Chibbar and R. B. van Huystee THE ROLE OF PEROXIDASE IN CELL DEVELOPMENT

Department of Plant Sciences
The University of Western Ontario

Peroxidase (E.C.1.11.1.7) isoenzymes have been used by plant scientists of diverse interests as marker of altered plant growth and development. Despite the numerous reports of its association with as diverse phenomena as salt tolerance to a change of sex in flowers, no precise function is attributed to this enzyme system. One of the main reasons for this situation is the indiscriminate use of peroxidase isoenzymes, without any regard to their real nature and biogenesis. The artifactuality of the anionic peroxidase isoenzymes was shown in our lab when the five anionic peroxidase isoenzymes from peanut cells were derived from one form by reaction with phenolics. Thus, for proper understanding of this enzyme system, the purification of this protein is necessary. Peroxidase is secreted by peanut cells in suspension culture, into the medium, which supports their growth. From this secreted peroxidase two major peroxidases, the cationic and an anionic form accounting for three-quarters and one-tenth of the peroxiase activity, respectively, of the medium were purified to apparent homogeneity. When these two purified molecules were assayed for the peroxidase and IAA-oxidase activity, no differences were found between the two. However, the importance of one form being secreted and not the other will be discussed in relation to their function.

Differing ontogenetic origin of PCR ('Kranz') sheaths in the leaf blades of C_4 grasses (Poaceae). Nancy G. Dengler[1], Ronald E. Dengler[2], and Paul W. Hattersley[3], [1]Department of Botany and [2]Scarborough College, University of Toronto; [3]Research School of Biological Sciences, Australian National University.

The origin and early development of the procambium and associated ground meristem of the major vascular bundles have been examined in the leaf blades of seven C_4 grass species, representing different taxonomic groups and the three recognized biochemical C_4 types (NAD-ME, PCK, and NADP-ME). Comparisons were made with the C_3 species, Festuca arundinacea (pooid). In NAD-ME (Panicum effusum, eupanicoid; Eleusine coracana, chloridoid) and PCK (Sporobolus elongatus, chloroidoid) species, the procambium of major bundles gives rise to xylem, phloem and a mestome sheath; associated ground meristem differentiates as PCA ("C_4 mesophyll") and the PCR ("Kranz") sheath. Development in the C_3 species parallels this pattern, except that associated ground meristem differentiates as mesophyll and a parenchymatous bundle sheath. In contrast, major bundle procambium of the NADP-ME C_4 species (Panicum bulbosum, and Digitaria brownii, eupanicoid; Cymbopogon exaltatus, andropogonoid) differentiates into xylem, phloem and a PCR sheath (a mestome sheath is absent); associated ground meristem gives rise to PCA tissue. These observations of major bundle development support W.V. Brown's hypothesis that the PCR sheaths of NAD-ME and PCK type C_4 grasses are homologous with the parenchymatous bundle sheaths of C_3 grasses, while in NADP-ME C_4 species they are homologous with the mestome sheath.

Pierre Fobert and David T. Webb, Department of Biology, Queen's University, Kingston K7L 3N6

Cytokinin Induced Shoot Formation With Lettuce Cotyledons

Cotyledons from eight lettuce (Lactuca sativa L.) cultivars were excised from five day old seedlings and placed on Murashique's Minimal Organic Media with or without cytokinins. On growth-substance free medium, root formation and callus initiation occurred with all cultivars, but shoot formation was never observed. At 2.3×10^{-6} M Kn, BA, or 2iP, shoot production was induced with all cultivars. However, different cultivars had diverse caulogenic, as well as rhizogenic and callogenic, potentials. When cytokinins were tested over a concentration range of 2.3×10^{-5} M to 2.3×10^{-8} M with Grand Rapid Lettuce, all growth-regulators elicited shoot formation. The optimal concentrations for organogenesis with 2iP, Kn and BA were 4.6×10^{-6} M, 2.3×10^{-6}M, and 4.6×10^{-7} M respectively. 2iP and BA produced more shoots than Kn at their optimal concentrations. BA was superior for callogenesis while 2iP was superior for rhizogenesis. Results provided here indicate that cytokinin-induced shoot formation, in the absence of auxins, is a wide spread phenomenon with lettuce.

Sclerotial development in two ectomycorrhizal fungi, Pisolithus tinctorius (Pers.) Coker & Couch. and Paxillus involutus (Batsch) Fr.

Grenville, D.J. and Y. Piché, Department of Botany and Genetics, University of Guelph, Guelph, Ontario N1G 2W1

The sclerotia of Pisolithus tinctorius form in an intercalary position on mycelial strands following ectomycorrhizal formation. Initiation involves numerous outgrowths of hyphal tips that branch frequently to form a tightly knit, lenticular swelling. Initiation of sclerotia occurs on mycelial strands that have turned bright yellow. Sclerotia are also yellow until they form a hard, black, smooth rind at maturity. SEM studies show that the developing sclerotia consist of interwoven, highly branched mycelia with the external hyphae possessing clamp connections and an extracellular coating. Light microscopy studies and histochemical investigations indicate that the sclerotia have an outer rind of thick-walled hyphae surrounding a medulla where lipid and other storage substances are found. Sclerotial development in Paxillus involutus is similar to that of P. tinctorius; however, formation is more rapid, sclerotia are spherical rather than lenticular and develop from buff brown mycelial strands and remain this colour until a black rind is formed at maturity. Sclerotia of P. involutus will also form on culture plates. Sclerotia of both fungal species are readily produced in growth pouches in association with Pinus strobus. It is possible that the sclerotia of these two species can be used as germplasm sources for further study of mycorrhizal systems and perhaps for inoculation of nursery stock. Research supported by a NSERC grant to R.L. Peterson.

I. Ockenden, Biology Dept., McMaster University, Hamilton, Ont. Measurement of Calcium in Cucurbita maxima and Cucurbita andreana embryos.

Dr. J.N.A. Lott's energy-dispersive x-ray analysis of globoid crystals within embryos of various species of Cucurbita showed that the smaller embryos, such as those of Cucurbita andreana, contained a high level of total calcium and had a uniform distribution of calcium within the embryo. The larger embryos, such as those of C. maxima, had a more localized calcium distribution and an overall lower level of calcium. The study of total calcium and calcium distribution pattern in the above species and their reciprocal hybrids offered a promising system to study some of the factors controlling calcium storage in the embryos. A number of techniques for mineral analysis were tested but all presented problems with the oily, high protein, high phytin-containing embryos. Results of the analysis of small and large embroys will be presented and the limitations of the technique used will be discussed.

Dayakar R. Pareddy, Department of Plant Sciences, University of Western Ontario.

In Vitro Culture of Field Corn Tassels.

The in vitro culture of corn tassels, intially reported for a sweet corn cultivar, has been extended to the field corn inbred cultivars Oh 43, and K-21. The conditions presently used to obtain optimum development include - increased sucrose levels (0.1 M to 0.3 M), the addition of 30 mg/l casein hydrolysate, non-shaking growth chamber conditions with an 18/6 hr. day/night cycle at 28°/24°C. Under these conditions, tassels, 1.0 to 1.5 cm at the time of culture, produce 2-400 mature spikelets containing anthers with mature pollen. Todate, a few pollen grains produced in this fashion have germinated on agar

A New Test for Cell Vitality

Carol A. Peterson and Janice M.C. Fischer,
Department of Biology, University of Waterloo,
Waterloo, Ontario

Calcofluor white M2R, a fluorescent fabric-brightener dye, has proved useful as an apoplastic tracer because it cannot penetrate the plasmalemmae of living cells. But when a cell dies, the semipermeable nature of its membranes is lost and the dye can diffuse into the protoplast where it causes the cytoplasm to fluoresce under ultraviolet light. In a heat-killed onion scale epidermal cell, the nucleus fluoresces more brightly than the cytoplasm but when the cells are killed by exposure to fixatives or heavy metals, nuclear fluorescence is less conspicuous. After the latter treatments, cell death could be detected in uniseriate layers of cells but only with difficulty in sections of multicellular organs. The dye provides an excellent test for cell vitality in animal cells. The use of nonpermeating dyes such as Calcofluor white or Evan's blue to establish cell vitality is preferable to the use of permeating dyes such as uranin (disodium fluorescein) or neutral red since some living cells will not accumulate the permeating dyes. Furthermore, when a nonpermeating dye is used, the protoplasts of living cells are unaffected and the same cells can be used in subsequent experiments.

V. R. Bommineni (U.W.O.)

Some preliminary observations on sex differentiation in maize ears cultured in liquid medium.

We present observations on successfully cultured corn ears which reveal some differential properties. When cultured at early stages primarily male flowers develop: When older ear primordia are cultured primarily female flowers develop. We also present observations which document the differential polypeptide synthesis associated with these differential paths.

EFFECTS OF SULPHUR DIOXIDE AND NITROGEN DIOXIDE ON INTUMESCENCE DEVELOPMENT ON LEAVES OF *SOLANUM TUBEROSUM* L.

J. M. Petitte and D. P. Ormrod
Horticultural Science Dept., Univ. of Guelph
Guelph, Ontario CAN N1G 2W1

Plants of *Solanum tuberosum* cvs. Superior, Norchip, Kennebec and Russet Burbank were propagated from green-sprouted tubers. Ten days after emergence, the plants were exposed to 0.11 ppm sulphur dioxide and/or 0.11 ppm nitrogen dioxide for 24 hours for 7 days in plexiglas exposure cubes. Forty-eight to 96 hours after the treatments had begun, intumescences were visible on the leaflets of the middle and upper canopies of 'Kennebec' and 'Russet Burbank' plants in all treatments except the SO_2 and NO_2 mixture. Intumescence injury was similar on both cultivars and began as interveinal greenish-yellow protrusions on the adaxial leaf surface. About 24 hours after becoming visible, the intumescences became brown and formed a rough surface. Epinasty occurred on the more severely injured leaflets. Intumescence was absent or visible on less than one percent of the leaflets in the Norchip and Superior cultivars. In a second experiment, plants of cvs. Kennebec and Russet Burbank, which were propagated from cuttings and exposed to the same treatments as described above, showed no intumescences, or had lesions on less than one percent of the leaflet surface after 7 days of exposure in all treatments.

Localization of RNA in corn root meristematic cells using RNase-colloidal gold complexes: An example of protein-gold labelling of plant tissues.

Piché, Y., R.L. Peterson and C.A. Ackerley, Department of Botany and Genetics, University of Guelph, Guelph, Ontario N1G 2W1

Colloidal gold marker systems based on the methodology described by Horisberger (1975) and Geoghegan and Ackerman (1977) are used extensively for the localization of various cellular substances. The majority of biological materials observed, however have been derived from animal tissues. In this study, RNase-gold complexes were applied to thin sections of glutaraldehyde-fixed and Spurr-embedded corn root tips in order to assess the specificity of these gold complexes for RNA in meristematic cells. Numerous micrographs showed that among cellular compartments, nucleoli, nuclei and portions of the cytoplasm were densely labelled whereas cell walls and vacuoles were infrequently labelled. A number of controls used to test the specificity of the labelling showed that RNase-gold was bound to RNA in the cells. Quantitative evaluation of the labelling performed on the samples using morphometric and X-ray microanalysis confirmed the distribution of RNase-gold based on visual evidence. These results show that corn root tissues fixed and embedded in this way retain RNA in a form which can be labelled with RNase-colloidal gold complexes. It should be possible to use colloidal-gold complexed to other proteins to localize various substrates in a variety of plant tissues.

J.-P. ZHAO. Biology Dept., McMaster University, Hamilton, Ont. Mitotic abnormalities induced in roots of *Z. mays* by MMS.

Methylmethanesulphonate (MMS) is an alkylating agent and a potent mutagenic agent. The response of maize to the related mutagen, ethylmethanesulphonate (EMS) has provided evidence that some cells complete one or more cell cycles before the EMS-induced lesion is fully expressed as a mutation or as a break: the response to MMS has been studied in order to determine whether mitotic cells of maize roots show a delayed response to the mutagen. Seeds were treated with 1 mM MMS for 1, 2 or 4 hr 24 hr from the start of inbibition. Mitotic aberrations were scored in roots at 72, 78, 96 and 120 hr. The aberrations included: 1) non-congression of chromosomes at metaphase; 2) delayed segregation of chromatids; 3) lagging at anaphase; 4) bridges and fragments. The 4 hr treatment was more effective in inducing aberrant mitoses than a 1 or 2 hr treatment but even with those treatments 2-7% of mitoses were abnormal at 120 hr, i.e. when cells were in the 2nd or 3rd cell cycle since treatment. The results show that MMS produces two changes, chromatid non-disjunction and breakage; that would result in aneuploidy for part of or for a whole chromosome. Both could produce phenotypic variegation of the type reported after EMS treatment: this evidence for a delayed response is similar to that found in *V. faba* after treatment with maleic hydrazide.

DIRECT AND INDIRECT EMBRYO GENESIS IN MICROSPORES OF ASPARAGUS OFFICINALIS L. CV VIKING

G.J. Rotondo, P.M. Harney, and H. Tiessen,
Department of Horticultural Science,
University of Guelph, Guelph, Ont.

Unincleate microspores cultured *in vitro* within intact asparagus ($2n = 20$) anthers were successfully diverted from their normal developmental pathway (microsporogenesis) towards direct and indirect (via callus) embryogenesis. Induction for both direct and indirect embryogenesis occurred with a frequency of 0.33% when cultured on a modified Murashige and Koog liquid medium. Cytological examination of the developmental pathways suggests that direct embryogenesis follows the B pathway whereas indrect embryogenesis follows the A-V pathway as proposed by Sunderland, 1974; 1980. Chromosome counts will be made to determine ploidy of the reqenerated plantlets.

D.WILSON. Biology Dept., McMaster university, Hamilton, Ont. Lesions Induced by Maleic Hydraxide Continue to be Expressed over Several Cell Cycles.

Seeds of *Vicia faba* were treated with 1mM maleic hydraxide (MH) for 2 hr, at 22 to 24 hr from the start of imbition. One advantage of this time of treatment is that ~90% of 1^{o} root meristem cells are in G_1: i.e. treatment affects a fairly homogeneous group of cells cf. the heterogeneous population of a growing root meristem which contains G_1, S, G_2 and mitotic cells. The second advantage is that there is a spread in the times cells take to complete their first cycle, i.e. from 50 to 70 hr. We can therefore compare breakage and reunion frequencies and the distribution of break in cells that took different times to complete the cycle in which they were treated with MH. Results: 1) the proportion of breaks that are $B^{"}$ and the proportion of $B^{"}$ undergoing sister-reunion increased between 58 and 72 hr.;2) at 72 hr, 19.8% of metaphases contained chromosome aberrations, showing that they were in the second mitosis (M_2) since treatment;3) of these M_2 cells, 13.7% also had chromatid changes, showing that MH-induced lesions may be expressed as chromatid breaks over at least two cell cycles. In some cells it is clear that a proportion of the lesions become aberrations in one cycle, while others become breaks in a subsequent cycle: thus lesions are not all expressed or repaired within the same cell cycle.

A PRELIMINARY REPORT ON WATER UPTAKE BY THE IMBIBING AND GERMINATING SEED OF TOBACCO (Nicotiana tabacum L.)

A. Walther and N. Rosa, Agriculture Canada, Research Station, Delhi, Ontario, N4B 2W9 (On leave from the University of Regina, Department of Biology).

The tobacco seed is nondormant. For germination it requires water, a physiological temperature range, and a minimal exposure to light. We determined water uptake to supplement other investigations using cv Delhi 76, 1978 crop. A mean air dry weight of ca. 80 ug and dimensions of the longest and shortest axis of about 0.7 and 0.5 mm are indicative of the size and shape of the average seed.

We have standardized a procedure for determining water uptake, defined as the increase in fresh weight from the start of imbibition at 25 C. Maximum water uptake occurs within the first hour of imbibition, followed by a very sharp decline during the second hour. Imbibition of the air dry seed is complete by 6 h, corresponding to a 25% increase in the fresh weight.

During the next 48 h period, the additional fresh weight increase approximates 10% only. Subsequently, water uptake is accelerating slightly, and is accompanied by the first noticeable change in the imbibed seed. This represents the transition from the imbibed to the germinating stage. Radicle elongation indicates successful germination, corresponding to a total fresh weight increase of about 70% by day four. Fresh weight determinations of empty seed coats indicate that a considerable proportion of the weight of the imbibed and germinating seed is due to water retained in the testa.

Rolf Rutishauser, McGill University, Montreal

Organization and development of the "leaf whorls" of Hydrothrix gardneri (Pontederiaceae)

RUTISHAUSER, R.: *Hydrothrix gardneri:* Bau und Entwicklung einer eigenartigen Pontederiacee. [*Hydrothrix gardneri:* Architecture and development of an extraordinary Pontederiaceae.] — Bot. Jahrb. Syst. 104, 115-141. 1983. ISSN 0006-8152.

Hydrothrix is a poorly known aquatic plant from eastern Brazil. The present study concerns the vegetative and floral morphology of this monotypic genus. The development of the peculiar leaf whorls, the sympodial shoot and the two-flowered inflorescences ("pseudanthia") is described. Arguments are offered supporting and refuting interpretation of the leaf whorls as representing short shoots with annular insertion along the long shoot (sensu GOEBEL 1913). An alternative interpretation is presented which considers the whorls to be composed of leaves of the same shoot order: the first of a whorl as an outgrowth of the shoot apex, the remaining whorl leaves as products of an intercalary meristematic ring below the shoot apex. The characters that *Hydrothrix* has in common with other Pontederiaceae, especially the *Heteranthera-Zosterella* group are listed. It is felt that *Hydrothrix* should be retained as an independent genus in the Pontederiaceae.

As a part of my new publication I will present the peculiar organization and development of the leaf whorls of this fully submerged water plant. It's also a good opportunity to plead for complementary views in interpretative plant morphology and morphogenesis.